Ctsl Cas9-KO Strategy

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Reviewer: Shuang Zhang

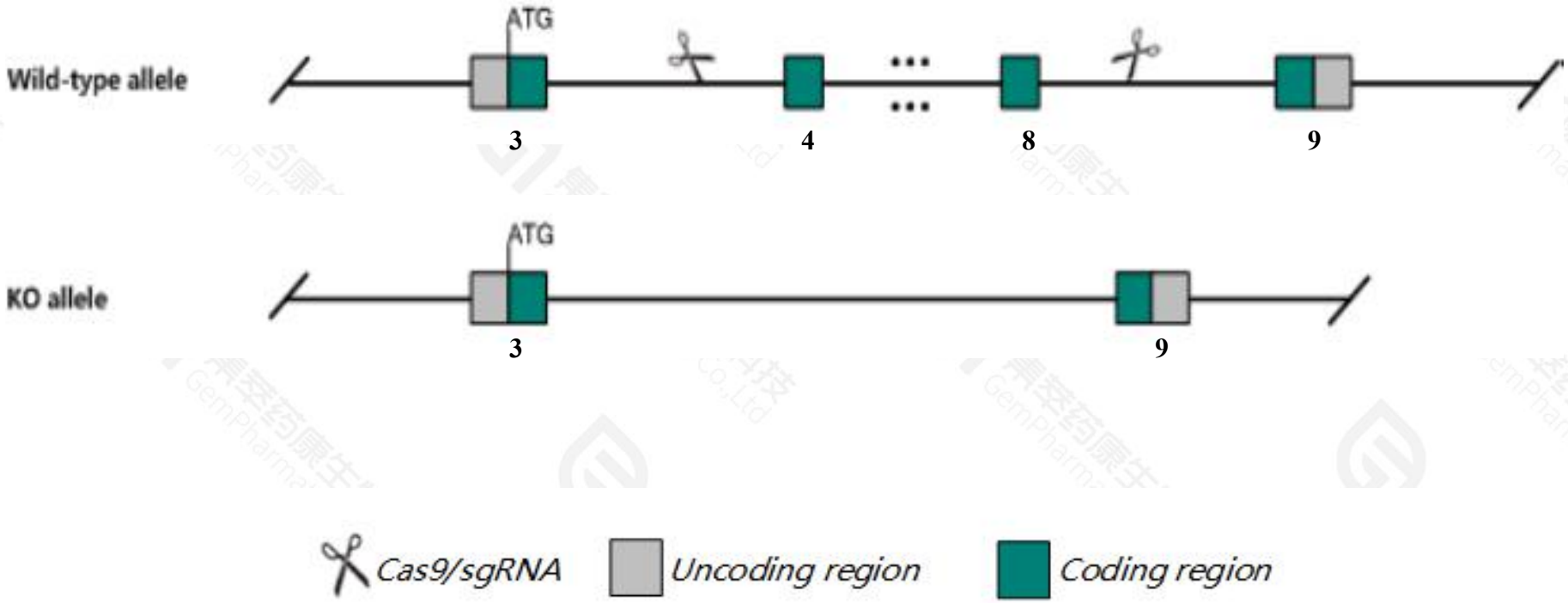
Design Date: 2021-7-15

Project Overview

Project Name	***Ctsl***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Ctsl* gene. The schematic diagram is as follows:



Technical routes

➢ The *Ctsl* gene has 7 transcripts. According to the structure of *Ctsl* gene, exon4-exon8 of *Ctsl-205*(ENSMUST00000222517.2) transcript is recommended as the knockout region.The region contains 776bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Ctsl* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA were microinjected into the fertilized eggs of C57BL/6JGpt mice.Fertilized eggs were transplanted to obtain positive F0 mice which were confirmed by PCR and sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6JGpt mice.

Notice

➢ According to the existing MGI data,homozygotes for mutant alleles may show partial or complete hair-loss, skin defects, impaired T cell maturation, dilated cardiomyopathy, and high postnatal mortality. Mutant males for some alleles show both normal and atrophic seminiferous tubules and reduced sperm production.

➢ The *Ctsl* gene is located on the Chr13. If the knockout mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes on the same chromosome.

➢ This strategy is designed based on genetic information in existing databases.Due to the complexity of biological processes,all risk of the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information （NCBI）

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Ctsl cathepsin L [Mus musculus (house mouse)]

Gene ID: 13039, updated on 12-Jan-2021

Summary

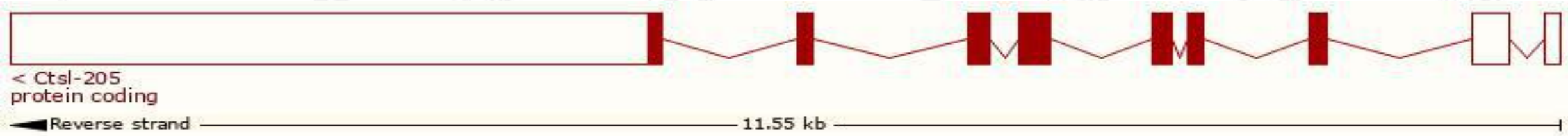
Official Symbol	Ctsl provided by MGI
Official Full Name	cathepsin L provided by MGI
Primary source	MGI:MGI:88564
See related	Ensembl:ENSMUSG00000021477
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	1190035F06Rik, CatL, Ctsl1, ME, MEP, fs, nkt
Summary	This gene encodes a member of the peptidase C1 (papain) family of cysteine proteases. The encoded preproprotein is proteolytically processed to generate multiple protein products. These products include the activation peptide and the cathepsin L1 heavy and light chains. The mature enzyme appears to be important in embryonic development through its processing of histone H3 and may play a role in disease progression in a model of kidney disease. Homozygous knockout mice for this gene exhibit hair loss, skin thickening, bone and heart defects, and enhanced susceptibility to bacterial infection. A pseudogene of this gene has been identified in the genome. [provided by RefSeq, Aug 2015]
Expression	Broad expression in placenta adult (RPKM 194.5), liver E18 (RPKM 75.5) and 20 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

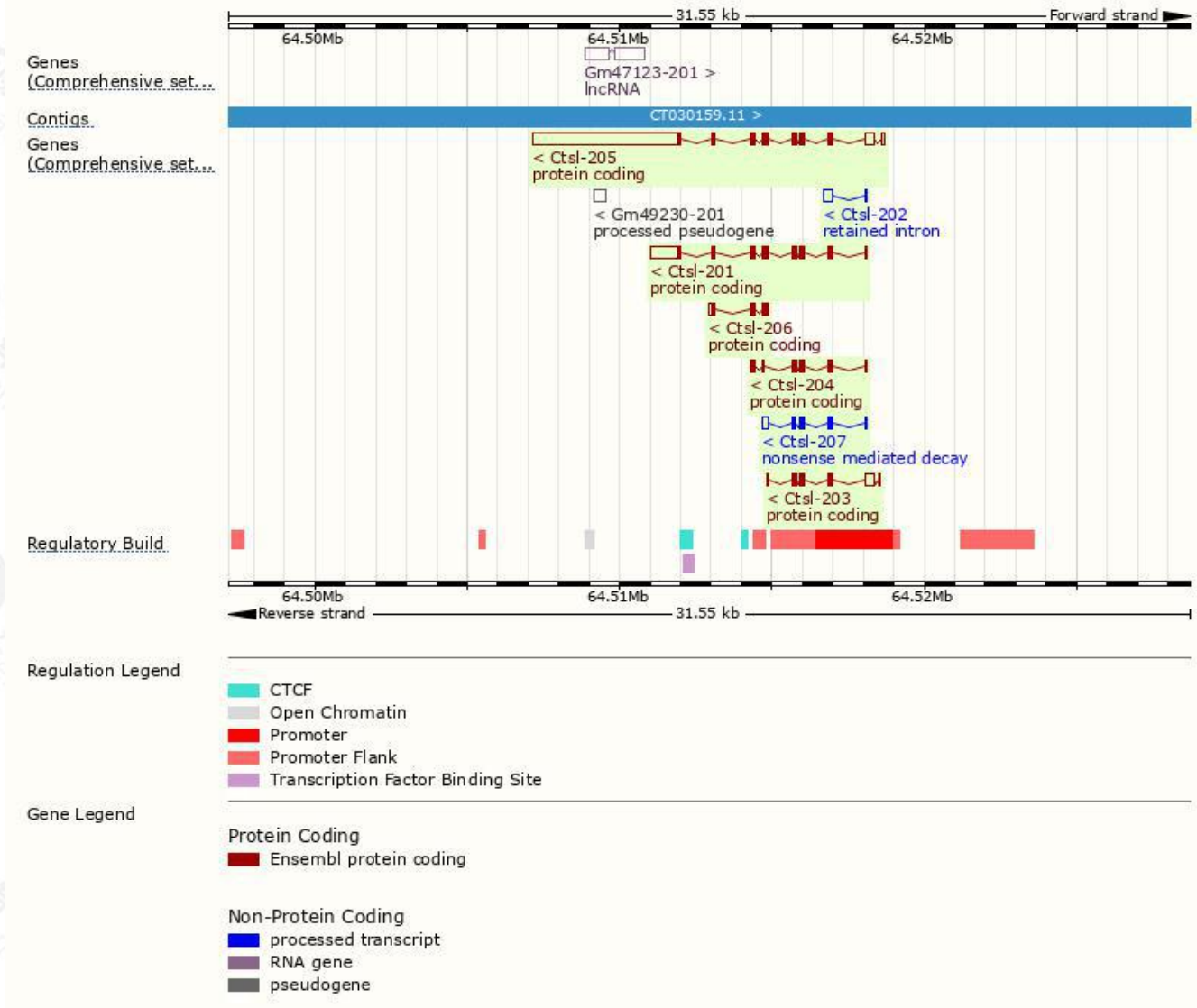
The gene has 7 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ctsl-205	ENSMUST00000222517.2	6168	334aa	Protein coding	CCDS26600		TSL:5 , GENCODE basic , APPRIS P1 ,
Ctsl-201	ENSMUST00000021933.8	1971	334aa	Protein coding	CCDS26600		TSL:1 , GENCODE basic , APPRIS P1 ,
Ctsl-203	ENSMUST00000220737.2	834	163aa	Protein coding	-		CDS 3' incomplete , TSL:5 ,
Ctsl-204	ENSMUST00000222462.2	698	205aa	Protein coding	-		CDS 3' incomplete , TSL:5 ,
Ctsl-206	ENSMUST00000222971.2	582	157aa	Protein coding	-		CDS 5' incomplete , TSL:1 ,
Ctsl-207	ENSMUST00000223494.2	644	94aa	Nonsense mediated decay	-		TSL:5 ,
Ctsl-202	ENSMUST00000220617.2	364	No protein	Retained intron	-		TSL:2 ,

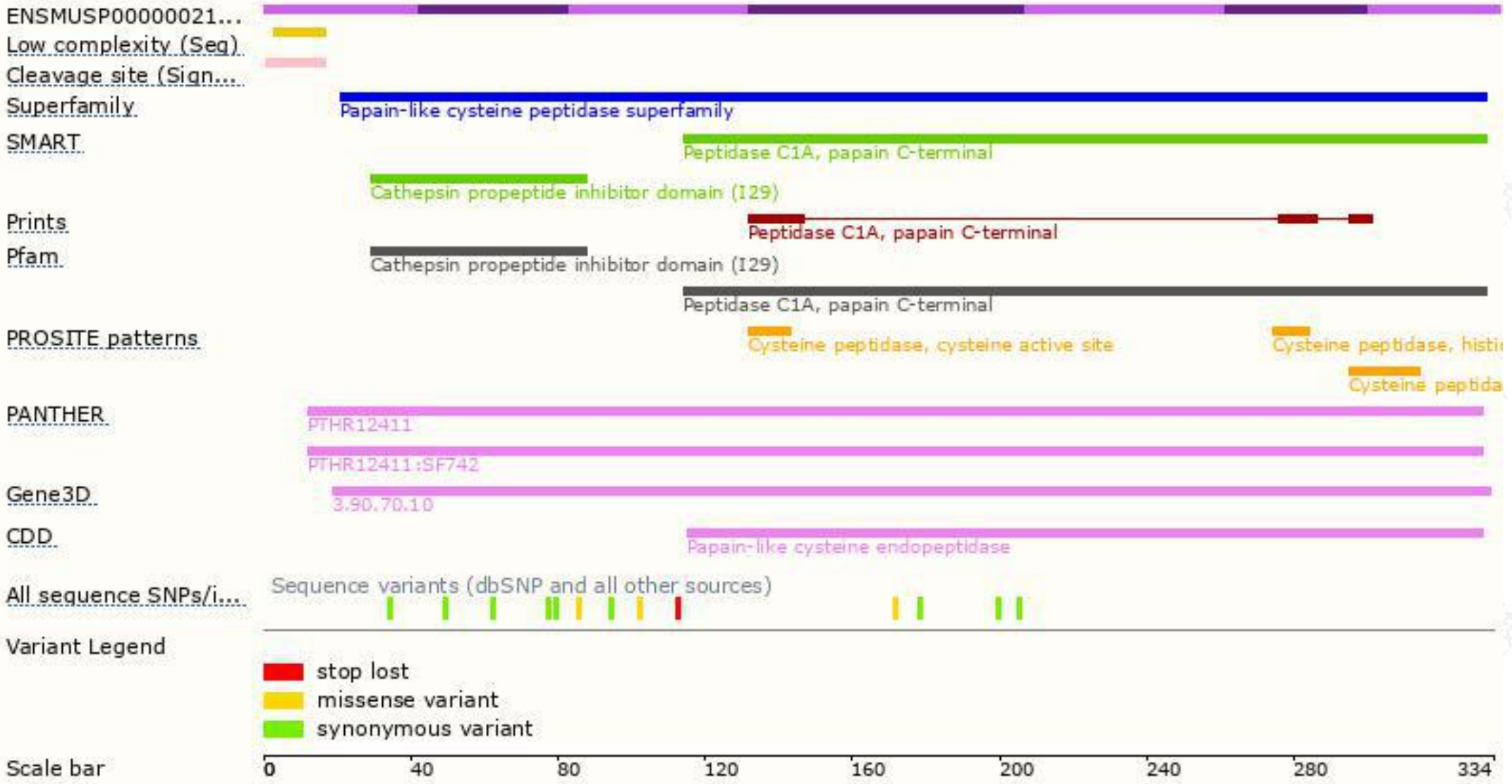
The strategy is based on the design of *Ctsl-205* transcript,the transcription is shown below:



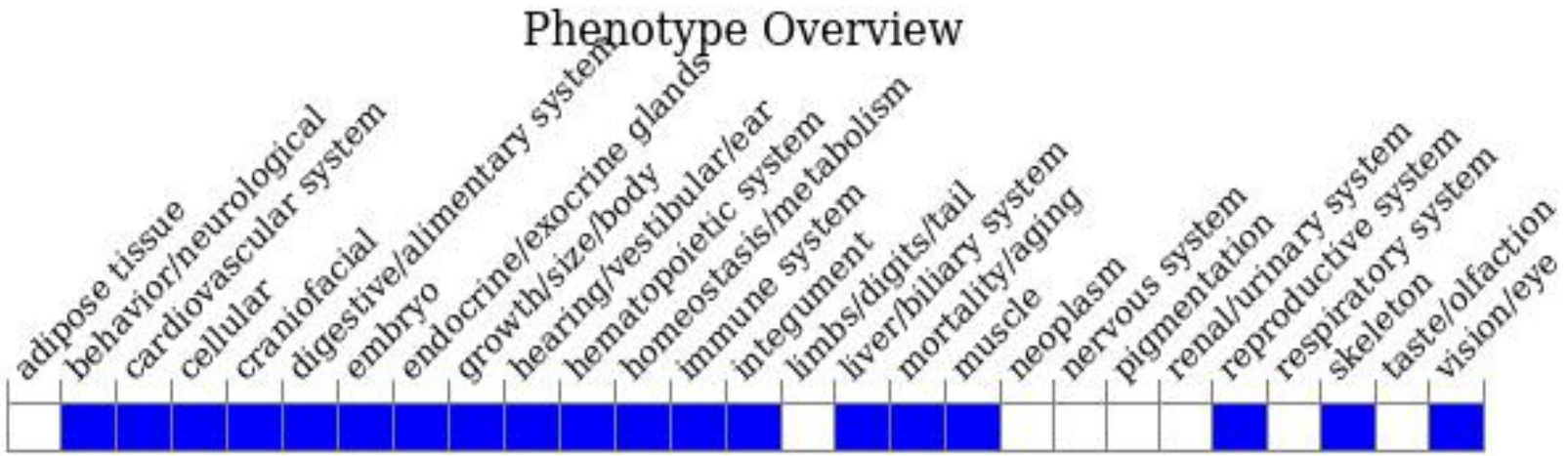
Genomic location distribution



Protein domain



Mouse phenotype description(MGI)



Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).

According to the existing MGI data,homozygotes for mutant alleles may show partial or complete hair-loss, skin defects, impaired T cell maturation, dilated cardiomyopathy, and high postnatal mortality. Mutant males for some alleles show both normal and atrophic seminiferous tubules and reduced sperm production.

If you have any questions, you are welcome to inquire.

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